BIOMATERIALS

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SILICON IN LIVING ORGANISMS AND NEW-GENERATION BIOCOMPOSITE MATERIALS (REVIEW)

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The effect of silicon compounds on the formation and growth of natural bone tissue is examined, and the concentrations of silicon compounds in different organ tissues are presented. The inductive action of different implantation substrates containing silica on the vital activity of cultures of osteogenic cells is described. It is suggested that the process resulting in bonding between bone tissue and silicate implantation material is affected by the presence of a substantial number of silanol groups on the surface of the material, which determine its high hydrophilicity. It is shown that the solubility of silicate glasses depends on the presence in them of a phase which corresponds to the composition of liquid glass, and its role in the biodegradation of the materials in physiological media is described.

Modern surgical methods of treatment in medicine are aimed not at removing a damaged organ bur rather to restoring its biomechanical properties. John Wolf's law (1872) — at the root of every regeneration lies nature's aim to restore not form but function — is the theoretical validation for the development of these directions in surgery and medical materials science. For bone – plastic surgery W. Roukes (1893) proved that bone possesses functional form and structure because of the functional stimulation of cells, which intensifies blood flow to the bone and results in its growth and, vice versa, decreased nutrition results in atrophy.

The advancement of restorative and replacement surgery became possible as a result of the development, first and foremost, of calcium – phosphate implantation materials which possess biological activity and stimulate the growth of bone cells. One such material is the porous apatite-silicate biocomposite BAK-1000, which as a result of the differentiated porous structure possesses osteo-conductivity [1]. Clinical experience gained over the last fifteen years of clinical experience in using implants based on the biocomposite BAK-1000 has demonstrated that this material is effective in neurosurgery and facial-maxillary surgery was well as in surgical stomatology. The advantage of this material over analogous calcium-phosphate composite materials is due to the presence in its structure of a hydrated silicate matrix, which makes its biological properties close to materials based on hydroxyapatite (HA) and bone protein — collagen [2].

However, the chemical – biological properties of the biocomposite material BAK-1000 and its modifications (BAK-1000 M, ORION-MB, BKS) in a number of cases are inferior to those of composites to which amorphous nanostructured HA has been added. In the present case, HA with 30 – 50 nm particles with amorphous crystalline structure in a hydrate shell possesses high chemical activity, which determines the effectiveness of such materials in stomatology (RF Patent No. 2245152) [3].

The biological role of phosphorus and its compounds in the formation of bone structures is not completely understood. However, silicon, which is a constituent of bioglass, bioceramics, glass ceramics, and biocomposite materials, is not given enough attention. In the present article, an attempt is made to examine certain aspects of the use of oxygen compounds of silicon in the synthesis of implantation materials and the participation of these compounds at the implant – bone interface.

Role of Silicon in the Formation of Skeletal Tissues. Silicon is the second, after oxygen, most abundant element in the Earth's crust (26%), and its crystalline form (quartz) is the most common mineral in the Earth's crust. The content and role of silicon in living systems is not the same. For example, V. I. Vernadskii divided living organisms into three categories based on this indicator: so-called silica organisms

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with more than 10% silicon by weight; silicon-rich organisms at least 1 – 2% silicon; and, ordinary organisms with only 0.1 – 0.001% silicon. In humans, silicon is a trace element and is measured in ppm (parts per million). For a long time, the biological role of silicon was not studied in Russia, because its content in the body's organs and tissues falls below the capabilities of existing analytical instruments. However, the understanding of biological processes in which silicon participates makes it possible not only to develop new-generation materials for implantation but also to predict the body's response to their implantation.

In animals and birds, silicon is present in the connective tissues, fur, nail plate, feathers, skin, tendons, muscles, and bones. In the human body the silicon content varies from 0.6 ppm in blood serum to 10 ppm in the liver and kidneys, 40 ppm in muscle tissue, 57 ppm in lung tissue, 100 ppm in bones, and 200 – 600 ppm in cartilage. Many investigations have shown that silicon is bound with a polysaccharide matrix and is present in the structure of glycosaminoglycan, polyuronide, hyaluronic acid, chondroitin sulfate, dermatin sulfate, and heparin sulfate. High concentrations of silicon are observed in intercellular matrix components, where silicon functions as a biological bonding agent capable of maintaining the architecture and elasticity of the connective tissues [4].

Silicon's role in physiological processes was first shown in [5]. It was determined that silicon is vital for normal growth and development of the skeletal tissues. At the stage of synthesis of collagen fibers and at the initial stages of biomineralization of bone silicon is associated with calcium, initiating the precipitation of bone minerals, being an important "transitional" element in the formation and development of cartilage and bone structures.

In vitro investigations using osteogenic cell cultures make it possible to predict the response of tissue under *in vivo* conditions to the placement of the implantation materials developed. The study of the behavior of osteogenic cells on the calcium-phosphate matrices modified by Na^+, K^+, and SiO_4^{4-} ions has shown that all substrates stimulated active growth of osteogenic cells. $CaNaPO_4$ and $CaNaPO_4$ with 9 wt.% SiO_2 promoted improved activation and differentiation of osteogenic cells as compared with the initial α tricalcium phosphate [6]. In [7] it is established that in the presence of the products of dissolution of bioactive glass BG60S the proliferation of cells increases by 35%, the viability of the cells increases, and the synthesis of collagen cells increases by 25% as compared with the two-phase calcium-phosphate ceramic. The authors of [8] also noted a substantial increase of the proliferation, differentiation, secretion of collagen and vitality of osteoblasts when the products of resorption of Bioglass glass and pseudo-wollastonite α-$CaSiO_3$ are introduced into the culture of osteogenic cells.

Numerous studies of the cultivation of osteogenic cells on various substrates have shown that attachment, proliferation, and differentiation of the cells and the synthesis of collagen and osteogenic markers all occur much more actively on materials which contain silicon compounds. It was found in [7] that the formation of a large number of vacuoles (75% of whose liquid contents consisted of silicon compounds) in osteoblasts had a positive effect on the viability and proliferation and synthesis activity of the cells. Such behavior can be explained, on the one hand, by the use of silicon as a "bonding" agent in the synthesis of collagen fibers by means of condensation reactions between colloidal silicon and collagen fibrilles and, on the other hand, by the additional adhesion interactions between the receptors of the cells and silica gel, formed on the surface of the substrate under the influence of the physiological medium.

Solubility of Bioactive Silicate Glasses *In Vitro* and *In Vivo*. Many investigations have now established that the solubility of bioactive silicate glasses *in vitro* and *in vivo* as well as their bonding with the skeletal and soft tissues is determined by the chemical composition of the glass and the contact medium.

Five regions of compositions with different biological activity have been found in the system $Na_2O – CaO – P_2O_5 – SiO_2$ [9]: nontechnical; absolutely inert; bioactive glasses bonding with bone; bioactive glasses bonding with bone and collagen fibers of soft tissues; and, biodegradable media whose resorption already starts during the first 10 – 30 days.

The behavior of bioactive glass of the system $SiO_2 – Na_2O – CaO – P_2O_5 – Al_2O_3 – B_2O_3$ was studied *in vivo* in [10]. The authors of [11] investigated glass of the system $SiO_2 – Na_2O – CaO – P_2O_5 – K_2O – Al_2O_3 – B_2O_3$ *in vitro*. In all works it is noted that for different compositions of the bioactive glasses the mechanism of the surface reactions and the formation of an implant – bone bond is the same. In all cases, the implant – bone concentration profiles showed that silicic acid gel forms on the surface of the implant under the influence of the physiological media. At the same time the content of the sodium, calcium, aluminum, and phosphorus content in the surface reaction layer is minimal. Calcium – phosphate layers of different "capacity" form on the surface of the silica gel.

The formation of the silicate gel is described by the reaction

$$\equiv Si – ONa + R – COOH \rightarrow \equiv Si – OH + R – COONa.$$

Osteoclasts effectuate resorption of implanted material. They attach themselves to the surface of the material, secrete and allot organic acids (citric, lactic, succinic, or carbonic) in the resorption zone. The soluble products of degradation enter the vascular system and are removed from the body [12].

Depending on the type of bioactivity of the glass (chemically stable, bioactive, biodegradable), the "thickness" of the silicic acid gel layer formed, and the flow the subsequent bonding reactions change. In the case of a chemically stable glass, the thickness of the silica gel layer formed is negligible; dissolution processes proceed much more actively and more deeply, forming a "thick" silica gel layer, on the surface

of the biodegradable glasses. The numerous polar groups ≡ Si – OH which are formed serve as adhesive islands, to which osteoblast cells attach by means of a condensation reaction between a protein molecule and a silanol group. The excess silanol groups undergo subsequent hydrolysis, which results in the formation of a highly hydrated colloidal silicic acid gel dissolved in the body's medium.

The degree of hydration of the surface of bioactive glasses is different and depends on the concrete composition — high-silica glass forms a strong continuous silicon-oxygen framework and is slightly subjected to hydrolysis. Likewise, glass with a low content of the silicon component and the CaO, P_2O_5, Al_2O_3, and B_2O_3, which stabilize the silicon-oxygen framework, hydrolyze weakly. Additions of Al_2O_3, Ta_2O_5, Sb_2O_5, TiO_2, and ZrO_2 inhibit bonding processes [9]. Conversely, increasing the Na_2O content at the expense of CaO holding the silicon and phosphorus oxides constant increases the solubility and results in the formation of a "thick" silica gel and gradual degradation of the implanted glass in the body's medium.

The mechanism of bonding of the bone tissue with the implanted material is similar to the mechanism of natural remodeling of bone [12]. Initially, the material is resorbed by osteoclasts, which can continue for up to six weeks in adults. The next phase is a reversion phase (1 – 2 weeks), which is characterized by a transition from resorption to formation of bone tissue as a result of the linking of the activity of osteoblasts and osteoclasts. The formation phase of osteogenesis starts with local differentiation of the pre-osteoblasts into osteoblasts and their migration into the region of the resorption lacuna. The pre-osteoblasts are located in the periosteum. The receptors of protein molecules in the cell membrane attach the osteoblast cells. On account of the high synthesis and secretion activity of osteoblasts the lacuna is gradually filled with organic intercellular matter (deposited at the rate 2 – 3 μm/day), and mineralization starts after 5 – 15 days; the entire process takes 20 weeks on average. Subsequently, the active osteoblasts lose their ability for secretion and to mineralize the bone matrix and transform into inactive osteoblasts.

Bioactive glass – bone bonds form via a condensation reaction between acidic ≡ Si – OH groups [1] and polar groups of adhesive protein molecules. Aside from the OH^- groups, sulfhydryl SH^-, carboxyl $-COO^-$, amino H_2N^-, and other groups are present in the protein molecules:

$$\equiv Si - OH + HO[C_nH_m] \rightarrow \equiv Si - O - [C_nH_m] + H_2O.$$

In addition, electrostatic bonds can appear between the protein molecules and the surface of the material. The strength of these bonds is negligible compared with valence bonds, but on account of their large number as well as the occurrence of the condensation reaction a protein molecule is quite strongly secured on the surface of bioactive glass.

After a bond forms between the bioactive glass and collagen the osteoblasts mineralize the osteoid section, which is formed, by synthesis and secretion of matrix bubbles containing alkaline phosphotase (in high concentrations). The micro-medium inside the matrix bubbles promotes deposition of the first HA crystals.

The bonding of the silicate gel and the formation of calcium-phosphate layers on its surface occur in two stages. First, silanol groups are bound with phosphate according to the neutralization reaction

$$\equiv Si - OH_{(gel)} + HPO^{2-}_{4(solution)} \rightleftarrows \equiv Si - O - PO^{2-}_{4(gel)} + H_2O.$$

The free chains ≡ Si – O – PO_2 – O are linked by calcium cations, which stabilize the silicate gel and form a three-dimensional structure:

$$\equiv Si_{(gel)} - O - \underset{\underset{O^-}{|}}{\overset{\overset{O}{\|}}{P}} - O - Ca - O - \underset{\underset{O^-}{|}}{\overset{\overset{O}{\|}}{P}} - O - Si \equiv_{(gel)}.$$

This mechanism decreases the atomic ratio Ca/P. *In vivo* investigations have confirmed that, ordinarily, at the initial stages of the formation of juvenile bone the values of the ratio Ca/P are low. Subsequently, low-cross-linked carbonate hydroxyapatite (CHA), in which the source of the CO_3^{2-} ions is, likewise, the bone tissue, crystallizes on the surface of the calcium-phosphate layer. Gradually, the ratio Ca/P increases, and then HA, carbonate hydroxyapatite, and tricalcium phosphate crystallize on the matrix. Ultimately, a strong bond forms between the bone tissue and the surface of the implant; if the implanted material is resorbed, the juvenile bone tissue gradually replaces the implant.

Glasses containing up to 55 wt.% SiO_2 and substantial quantities of univalent modifying oxides are prone to biodegradation. Such glasses are bioactive; bone tissue bonds to their surface by a number of chemical reactions, whose rate is quite high. In glasses containing more than 55% SiO_2, the bonding rate decreases substantially, and the material corresponds to an isobioactive state. The reaction of the bone tissue on such materials reduces only to limited bonding with the surface layers, the reactions do not occur in deep layers, and the material does not undergo any changes over time. When the content of the silicon component of the glass increases to more than 60%, the bonding of the bone tissue with the surface of the material is minimal, and the material is characterized by bioinert behavior.

The solubility of bioactive and biodegradable glass in the system Na_2O – CaO – SiO_2 is due to the presence of sodium and potassium silicates, which form the compounds $yNa_2O \cdot zCaO \cdot xSiO_2$ in the glass. It is well known that glasses with the composition $Na_2O \cdot xSiO_2$ are liquid [13], so that in a biodegradable glass this is the first component to dissolve. The high reactivity of $Na_2O \cdot xSiO_2$ compounds

shows that sodium and potassium silicates are not found in the Earth's lithosphere, but their compound with aluminum results in the formation of sodium and potassium feldspars — albite $Na_2O \cdot Al_2O_3 \cdot 6SiO_2$ and orthoclase $K_2O \cdot Al_2O_3 \cdot 6SiO_2$, which exhibit adequate stability. The study of the properties of liquid glasses makes it possible to understand the nature of the processes occurring in the physiological medium when bioactive and biodegradable glasses are implanted.

It is known that liquid glasses are capable of transitioning from the solid into the liquid phase in atmospheric air by means of hydration:

$$Na_2O \cdot xSiO_{2(solid)} + CO_{2(air)} + NH_2O \rightarrow Na_2CO_{3(solution)} + xSiO_2 \cdot NH_2O_{(liquid)}.$$

The glassy alkali silicates can dissolve without changing composition only in water, and substantially soluble liquid glass can form transparent gelatin-like gels. In reactions of liquid glass, hydrolysis of the embryonic nuclei obtained as well as their colloidal nature and high adsorption power are of great significance.

Liquid glass is capable of emulsifying organic binding materials well. Organic compounds which are insoluble in water engender coagulation of the liquid glass.

Alkali silicates are salts of a very weak silicic acid, so that all soluble mineral and organic acids will displace it from all of its salts. The decomposition of liquid glass by all acids proceeds via the same scheme with formation of a water soluble alkali salt corresponding to the acid and ultimately with the precipitation of a silica gel residue.

The oxides and hydroxides of alkali-earth metals interacting with liquid glass form colloidal residues of silicates of alkali-earth metals:

$$Na_2O \cdot xSiO_{2(liquid)} + Ca(OH)_2 \rightarrow 2NaOH + (x-1)SiO_2 + CaSiO_{3(colloid.)}.$$

Silicic acid gel obtained under the action of physiological liquids can be regarded as a soluble form of silicates, similar to liquid glass. It is the silica gel that enters into reaction with calcium ions, forming a colloidal gel of calcium silicate, which subsequently interacts with soluble phosphates, promoting the formation of calcium – phosphate layers observed in all concentration profiles *in vitro*. On account of the high adsorption characteristics and substantial hydrophilicity, many proteins, growth-factor molecules and protein and collagen molecules which effectuate the implant – bone bond can attach to its surface. The silicates and silica gel easily adsorb various organic compounds ranging from alcohols, glycols, and amino acids to sugars and nucleic acids. Vitamins and lipids are also well adsorbed on silica gel. Silica gel adsorbs water to a substantial degree, and undergoes gradual biodegradation and is completely removed naturally.

The basis for understanding the structural-chemical structure of bioactive glasses and other glass materials as well as silica gels as they degrade in physiological media is information on the short-range order in them, i.e., on the elementary coordination anionic groups. The values of the intensity F of the ionic field of certain elements which are present in various inorganic bioactive materials are given in Table 1 as a function of the charge Z and ionic radius r: $F = Z/r^2$. It follows from these data that as the intensity of the ionic field increases, the acidic properties of the cation become stronger and, conversely, as the field intensity decreases, the basic properties become stronger.

TABLE 1.

Cation	Ionic radius, Å	Field intensity, $Å^{-2}$	Chemical character
B^{3+}	0.20	75.0	Acidic
P^{5+}	0.34	43.2	
Si^{4+}	0.41	23.8	
Ge^{4+}	0.53	14.2	
Al^{3+}	0.31	12.0	Acidic – basic
Ti^{4+}	0.50	8.7	
Zr^{4+}	0.80	6.3	
Mg^{2+}	0.65	4.7	Basic
Ca^{2+}	0.99	2.0	
Na^{+}	0.95	1.1	
K^{+}	1.83	0.6	

On the basis of general thermodynamic ideas it can be supposed that various polymer groups formed according to the following reactions and differentiated according to composition and structure are present in implanted material and in the surface layer of the silica gel:

association – polycondensation of elementary ion groups on interaction of polar groups – OH;

acid – base neutralization reaction of glass-forming anions with uni- and bivalent alkali ions; and,

according to the type of solid solutions, homo- and heterovalent substitutions in anionic structural groups with excess negative charge.

These reactions result in the formation of a nanostructured amorphous-crystalline gel, comprising a 150 – 300 nm thick layer, depending on the degree of surface degradation of the material. Low-angle x-ray diffraction data show the chemical nonuniformities to be 10 – 15 nm in size [14].

Such differentiation of the surface material according to structure and composition promotes the formation of strong chemical bonds between the collagen protein and the implant.

Excretion of Silicon Compounds from the Body. Many investigations have shown that bone tissue is capable of binding with bioactive glasses which promote bone growth and engender an unknown immune response.

The authors of [4] examined in detail the excretion of silicon by experimental animals after implantation of bioactive glass BG into bone tissue. Granules of the bioactive glass

BG, which were 300 – 335 μm in size, were implanted in the proximal femur of rabbits in the first group. Over 28 weeks measurements the rate of excretion of silicon from the body of the experimental animals was evaluated. For this, measurements were performed of the content of this element in urine and blood. At the end of one week the animals were euthanized and the content of silicon in the kidneys, liver, lungs, spleen, and lymph system and at the implantation site — the proximal femur — as well as in the distal femur was studied. The data on the rate of excretion of silicon for animals in the first group were compared with the results obtained similarly for silicon excretion for animals in the second group which were not subjected to the bioactive glass implantation operation.

It was established that the degrading material remains in the implantation zone for a short time. The rate of dissolution of the silicon component of the bioactive glass is lower than the rate of removal of the silicon through the kidneys and, correspondingly, silicon does not accumulate in the body. The results showed that for the group-I animals silicon did not accumulate in the body, the total content of silicon in the blood of the kidneys, liver, lungs, lymph system, and spleen was at the level of silicon in the group-II animals, but a negligible amount of silicon was observed to accumulate in the distal femur. All silicon left the bone tissue in 24 weeks, and total resorption of bioactive glass from muscle tissue occurred over 19 weeks.

A solid trend toward developing implantation materials whose chemical composition and differential structure of the surface layers correspond best to the character and sequence of biochemical reactions in contact with living bone tissue has now emerged in world practice and in Russia. Regeneration of bone tissues can be stimulated when bone cells are actively influenced as well as when nanostructures of the chemical compounds participating in these reactions are present in the material. The data examined show that it would be useful to develop and apply silicon-containing implantation materials based on tricalcium phosphate, calcium hydroxyapatite, carbonate hydroxyapatite, and other calcium phosphates.

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